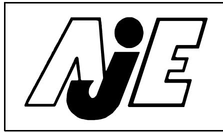

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HUMAN GENOME EPIDEMIOLOGY (HuGE) REVIEW

CYP3A4 Polymorphisms—Potential Risk Factors for Breast and Prostate Cancer: A HuGE Review

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The steroid hydroxylase CYP3A4 is the most abundant P-450 enzyme in the human liver, and CYP3A enzymes metabolize more than 50% of prescription drugs. The *CYP3A4* gene is expressed in the liver, gut, colon, prostate, and breast. Individual variation in CYP3A4 may play a role in breast and prostate carcinogenesis through modulation of sex hormone metabolite levels. Alternatively, CYP3A4 can metabolically activate exogenous carcinogens. CYP3A4 activity varies widely in humans, and more than 78 DNA sequence polymorphisms are known. These observations prompted the hypothesis that variant *CYP3A4* may be involved in breast and prostate cancer. Two epidemiologic studies of breast cancer and five of prostate cancer examined *CYP3A4* genotypes. A US study showed that inheritance of *CYP3A4*1B* correlates with early menarche, a breast cancer risk factor. However, an Australian breast cancer case-control study found no association with *CYP3A4*1B*. Two Scottish prospective studies showed *CYP3A4*1B* to be a risk factor for prostate cancer among men with benign prostatic hyperplasia. Three other studies were undertaken in the United States: two were case-only studies and the other was a case-sibling control study. Although results for African Americans were inconsistent, these studies suggested that *CYP3A4*1B* was associated with markers of advanced disease. These observations support the notion that development of robust, conventional molecular epidemiologic case-control studies to address these questions, including gene-gene and gene-environment interactions, will be timely.

breast neoplasms; *CYP3A4*; cytochrome P-450 enzyme system; epidemiology; genetics; hormones; metabolism; prostatic neoplasms

Abbreviations: CI, confidence interval; *CYP3A4*, the gene coding for the cytochrome P-450 enzyme designated CYP3A4; OR, odds ratio; PCR, polymerase chain reaction; UTR, untranslated region.

Editor's note: *This article is also available on the website of the Human Genome Epidemiology Network (http://www.cdc.gov/genomics/hugenet/reviews.htm).*

GENE

Cytochrome P-450 genes constitute a superfamily coding for different isozymes that account for phase I drug metabo-

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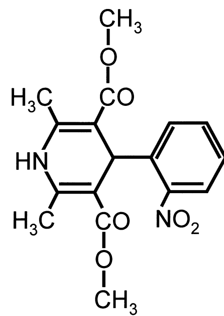

FIGURE 1. The chemical structure of nifedipine.

lism. In humans, more than 40 different cytochrome P-450s have been identified and sequenced (1–3). CYP3A4 was originally named nifedipine oxidase for its ability to metabolize the antianginal drug nifedipine (figure 1). However, since cytochrome P-450 substrate specificities proved to be broad and overlapping, a formal evolutionary nomenclature was defined that more aptly categorizes each isozyme. CYP denotes cytochrome P-450 for humans (cyp for mouse). The gene families are then designated by numbers following CYP. Originally, these were Roman numerals but were changed to Arabic. Subfamilies are represented by a letter followed by a number for the individual gene (4, 5). For example, for *CYP3A4*, 3 denotes the gene family, A the subfamily, and 4 the gene coding for a specific polypeptide. Current estimates indicate that each mammalian species may have between 40 and 200 distinct functional cytochrome P-450 genes (4, 6).

The *CYP3A4* gene, located on chromosome 7q21.3-q22.1, is 27,592 base pairs long and has 13 exons. The promoter region encompasses a basal transcription element (–35 to –50). Also present in the 5′ untranslated region (UTR) are an AP-3 binding site, a p53 binding motif (a specific DNA sequence to which the protein p53 can attach), a hepatocyte nuclear factor-4 element, two hepatocyte nuclear factor-5 elements, a glucocorticoid response element, and an estrogen response element (7, 8). The corresponding protein is also known as CYPIIIA4/nifedipine oxidase/NF-25/P-450-PCN1 and consists of 502 amino acids with a molecular weight of 57.29 kDa. It is membrane bound and is present in endoplasmic reticulum. Further information concerning its structure and location can be obtained at the following website: http://www.ncbi.nlm.nih.gov/htbin-post/Omim/getmap?l124010.

CYP3A4 is expressed in the prostate, breast, gut, colon, and small intestine, but its expression is most abundant in the human liver, accounting for 30 percent of the total CYP protein content (9–14). It exhibits a broad substrate specificity and is responsible for oxidation of many therapeutic drugs and a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics. In liver microsomes, it is involved in a nicotinamide adenine dinucleotide phosphate-dependent electron transport pathway. Pertinent to this review, CYP3A4 has an important role in the oxidation of both testosterone (2β-, 6β-, or 15β-hydroxytestosterone) and estrogen (4- and 16α-hydroxylation) (15–18). It can be induced by various compounds including drugs, pesticides, and carcinogens, resulting in high CYP3A4 levels in liver and other tissues, including mammary. A mutation in *CYP3A4* may lead to a reduced potential for oxidizing testosterone, leaving a greater bioavailability of the hormone to be metabolized intracellularly to its biologically active form of dihydroxytestosterone, the principal androgenic hormone involved in regulating prostate growth (figure 2).

GENE VARIANTS

Prevalence

Ovid software (Ovid Technologies, Inc., New York, New York) was used to search the MEDLINE database (National Library of Medicine, Bethesda, Maryland) between January 1, 1989, and May 3, 2004, using the search terms cytochrome P-450, breast neoplasms, and prostate neoplasms. The GenBank resource at the National Center for Biotechnology Information of the National Institutes of Health (Bethesda, Maryland) (http://www.ncbi.nlm.nih.gov/Genbank/GenbankSearch.html) was used to search for DNA sequence information. Accession numbers for gene sequences are those of the National Center for Biotechnology Information. Numerical locations of polymorphisms

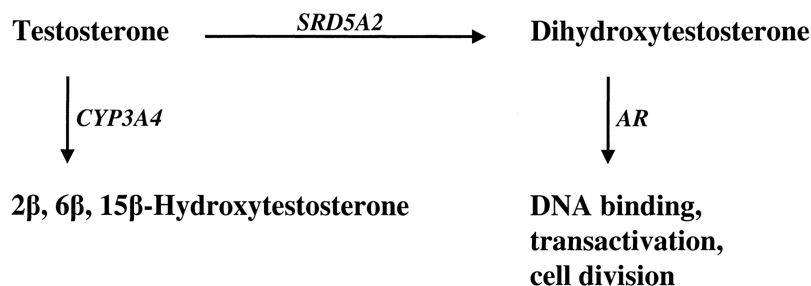

FIGURE 2. *CYP3A4* and androgen metabolism. *CYP3A4* is associated with oxidative deactivation of testosterone and is responsible for regulation of testosterone's metabolism to 2β-, 6β-, and 15β-hydroxytestosterone, less biologically active forms of testosterone. *SRD5A2*, steroid-5-α-reductase gene; *AR*, androgen receptor gene.

TABLE 1. DNA sequence variants of *CYP3A4* found in the enhancer region, promoter region, and 3′UTR*

Cytochrome P-450 nomenclature	Distance from the ATG signal†	Position in GenBank sequence AF280107	Change and sequence context
*CYP3A4*1A*	1	62037	aaagtagtgAtggctctcat
*CYP3A4*1u*‡	7904§	54133	ttgattatC/Aaaagaaact
*CYP3A4*1v*‡	7601	54436	ttaaaaaaaA/-tgttactag
*CYP3A4*1B*	392	61645	agggcaA/Ggagagag
*CYP3A4*1C*	444	61593	ggcttgtT/Ggggatgaa
*CYP3A4*1D*	62	61975	gcccagC/Aaaagagca
*CYP3A4*1x*	66	61971	gcacatagcC/Gcagcaaaga
*CYP3A4*1M*	156	61881	gctgcagctC/Acagccctgc
*CYP3A4*1E*	369	61668	aatagattT/Atatgccaa
*CYP3A4*1w*	666	61371	gaaacaggcG/Atggaaacac
*CYP3A4*1K*	655	61382	tggaaacacaA/Gtggtggtaa
*CYP3A4*1L*	630	61407	aagaggacaaA/Gtaggattgc
*CYP3A4*1F*	747	61290	acagcacC/Gctggtagg
*CYP3A4*15B*¶	844^845#	61191^2	aagATGGAGTGAtca
NFSE*	246^247	61789^90	agtgagtgGTgtgtgtgtg
	219	61818	ccaacttccA/Caggtgga
	120	61917	aaacaatccA/Gacagcct
	34	62003	aaaggaagA/Tctcagagg
3′UTR	25958	87994	taaggacttcT/Ggctttgct
*CYP3A4*1aa*	26011	88049	aaattacttT/Ggtgaataga
*CYP3A4*1T*	26011	88049	aaattacttT/Cgtgaataga
	26082**	88120	ttctgtacaT/Ggcattgagc
	26082††	88120	ttctgtacaT/Cgcattgagc
*CYP3A4*1H*	26204	88242	tccaccaccC/Accagttagc
	26269	88305	tcaataatttC/Tctccacaa
	26418	88454	ctttcctgcaC/Tattaagga

* UTR, untranslated region; NFSE, nifedipine-specific responsive element.
† In GenBank sequence AF280107, the A in this signal is located at nucleotide number 62037.
‡ This haplotype occurs together with the G/A single nucleotide polymorphism at 82266 (refer to the footnotes to tables 2 and 3).
§ These numbers are expressed as negative in the text because these positions precede the start signal.
¶ A variant of *CYP3A4*15A*.
# The chevron indicates a nucleotide insertion.
** Data from Dai et al. (35).
†† Data from Lamba et al. (30).

are given as distances in base pairs from the methionine translational start signal (ATG), where A is nucleotide +1 and its adjacent 3′ nucleotide is –1. The A in the ATG site for *CYP3A4* can be found at position number 62037 in the GenBank entry identified by accession number AF280107.

Allelic frequencies and their 95 percent confidence intervals were determined according to standard methods by using Microsoft Excel software (19). Allele frequencies were used to calculate the expected numbers of persons with a given genotype according to Hardy-Weinberg population laws. Chi-square statistics were used to determine whether experimental observations departed significantly from Hardy-Weinberg equilibria (20). Crude odds ratios were calculated according to the Mantel-Haenszel method (21) by using SAS statistical software (22).

Discovery of CYP3A4 *gene variants.* Although human interindividual variation in 6β-hydroxycortisol:cortisol ratios suggested their existence, genetic polymorphisms of *CYP3A4* were unknown until 1996 (23). Since 1998, at least 78 nucleotide sequence variations of *CYP3A4* have been identified (tables 1, 2, and 3). Most of the variants are correctly characterized as polymorphisms (frequency ≥ 0.01, at least 1 percent of the chromosomes in a given population), whereas many are technically constitutive

TABLE 2. DNA sequence variants of *CYP3A4* found in the coding region

Cytochrome P-450 nomenclature*	Distance from the ATG signal†	Position in GenBank sequence AF280107	Change and sequence context	Amino acid change	Exon
*CYP3A4*14*	44	62080	gcttctccT/Cggctgt	L15P	1
*CYP3A4*7*	6004	68040	tcccaggG/Acttttgt	G56D	3
*CYP3A4*4*	13871	75907	aagtgccA/Gtctctat	I118V	5
*CYP3A4*8*	13908	75944	agattacG/Aatcattg	R130Q	5
*CYP3A4*15A*	14269	76305	tctgaggcG/Aggaag	R162Q	6
*CYP3A4*9*	14292	76328	gcaagcctG/Atcacct	V170I	6
*CYP3A4*10*	14304	76340	ccttgaaaG/Cagtaag	D174H	6/7
*CYP3A4*16*‡	15603	77639	tgtgatcaC/Gtagcac	T185S	7
*CYP3A4*17*	15615	77651	cacatcatT/Ctggagt	F189S	7
*CYP3A4*5*	15702	77738	ttttggatcC/Gattcttt	P218R	7
*CYP3A4*2*	15713	77749	attctttctcT/Ccaataa	S222P	7
*CYP3A4*6*	17662^3§	79698^9	gatgattgac∧Atctcag	277∧S278I (285^{Stop})¶	9
*CYP3A4*18*‡	20070	82106	ctgtccgatcT/Cggag	L293P	10
*CYP3A4*11*‡	21867	83903	gaatgaaaC/Tgctcaga	T363M	11
*CYP3A4*12*	21896	83932	gctatgagaC/Tttgaga	L373F	11
*CYP3A4*13*	22026	84062	agttcctccC/Ttgaaagg	P416L	11
*CYP3A4*3*	23172	85208	gcattggcaT/Cgaggttt	M445T	12
*CYP3A4*19*#	23237	85273	tccttcaaaC/Tcttgtaa	P467S	12
Trivial**					
CYP3A4$^{682C/T}$	15628	77664	gagtgaacatC/Tgactct	I193I††	7
	20083	82119	ctcgtggcC/Tcaatcgaa	A297A	10
	21817	83853	ccacccacC/Atatgata	T346T	11
M15	21868	83904	tgaatgaaacG/Actcaga	T363T	11
M18	25925	87961	gggatggcacC/Tgtaagt	T499T	13

* Refer to the Human Cytochrome P450 (*CYP)* Allele Nomenclature Committee website (http//www.imm.ki.se/cypalleles/).

† Relative to A at nucleotide 62037 in GenBank sequence AF280107.

‡ Haplotypes *CYP3A4*11b*, **11c*, **16B*, and **18B* have been described recently (24).

§ The chevron indicates a nucleotide insertion.

¶ The A insertion causes a frame shift, and a stop codon results 7–8 codons downstream.

Refer also to table 3, footnote §.

** Names given in original publications.

†† Silent changes.

mutations (frequency ≤ 0.01). Some genetic variants are named according to the scheme outlined above; some have trivial names. However, it is important to recognize that haplotypes comprising multiple polymorphisms, or mutations, have barely been recognized. Originally, three pairwise haplotypes were described: *CYP3A4*15A*, *CYP3A4*15B*, and *CYP3A4*19*. Most recently, a Japanese consortium released details of 25 haplotypes, including 17 novel DNA-sequence variants (24). Note that several of these haplotypes are not yet unambiguous, and some may be limited to this population. In addition, a recent study described haplotypes between *CYP3A4*1B* and *CYP3A5*1* as well as *CYP3A4*1B* and *CYP3A5*3* in prostate cancer (25).

A study conducted at the Hospital of the University of Pennsylvania, Philadelphia, specifically to identify *CYP3A4* polymorphisms evaluated a panel of DNA samples assembled from buccal swabs of 94 Caucasian male volunteers with no history of cancer (26). A single nucleotide polymorphism was identified in a 5′ regulatory element of *CYP3A4* by using conformation sensitive gel electrophoresis and direct DNA sequence analysis. This single nucleotide polymorphism (A/G) at nucleotide position –392 is found in the nifedipine promoter region. The common variant was designated *CYP3A4*1A*, and the newly discovered minor variant was designated *CYP3A4*1B* (table 1). This polymorphism was independently identified by direct DNA sequencing and gel mobility shift assay of liver DNA obtained through either

TABLE 3. DNA sequence variants of *CYP3A4* found in introns

Designation*	Distance from the ATG signal†	Position in GenBank sequence AF280107	Change and sequence context	Intron
*CYP3A4*1y*	3856	65993	tatctataaA/-gtcacaatc	1
T5922C‡	5917	67953	tctgatttcaT/Ctggcttcg	2
*CYP3A4*1J*	6076	68113	gaaacttccA/Gttggataga	3
T6165A	6159	68195	gggatgaagctcT/Atgtca	3
SNP4	13804	75840	ccacaactgA/Ttgtaggaca	4
G13875A	13757	75793	tgaataagtG/Attcctgttta	4
G13947C	13829	75865	tgtttctgctttG/Caactctag	4
SNP5	14200	76236	tatgggtggtgT/Gtgtgtttt	5
M10	14323	76359	aagcgcagcC/Tatggggtt	6
M11	14329	76365	gccatgggG/ttctgagctgtc	6
T14475G	14357	76393	cccctccagcT/Ggcctgcca	6
*CYP3A4*1z*	15552	77589	taattttccA/Ccatctttct	6
*CYP3A4*1P*	15726	77763	aagtatgtgG/Aactactatt	7
T15871G	15753	77789	ttttatttatcttT/Gctctcttaaa	7
T15901C	15783	77819	tttattgagaT/Cataaatcacca	7
SNP10	15804	77840	tgtaattcaT/Gccacttaaaa	7
*CYP3A4*1Q*	15808	77845	ttcatccacT/Ctaaaatata	7
T15955A	15837	77873	gtgatttgtagT/Aacatttgaag	7
*CYP3A4*1R*	16774	78811	acattcacaA/Gtgaatttct	7
C17141T	17024	79060	gtgcagttacC/Ttgtatgttttta	8
*CYP3A4*11b*	17717	79754	tttctgaggG/Actacttgtg	9
SNP14	17815/6	79851/2	agaacgacacAT/-gtttgaat	9
G20338A§	20230	82266	tgagtggatgG/Atacatggag	10
SNP16	20265	82301	GaaaccttagC/Taaaaatgcc	10
G20417C	20309	82345	tttttataaaaaG/Ccataatcact	10
A21891C	21785	83821	caatttatccaA/Catctgtttcgt	10
SNP18	21795	83831	caaatctgtttcG/Attctttccagg	10
M16	22041	84077	aggtacaaggC/Tccctgggaa	11
SNP21	23024	85060	aagtaagaaA/Gccctaacatg	11
C23187T	23081	85117	aaaaatctaccaaC/Tgtggaac	11

* Trivial names given in original publications, unless italicized.
† Relative to A at nucleotide 62037 in GenBank sequence AF280107.
‡ The four- and five-digit designations are reportedly from GenBank sequence AF209389 (Lamba et al. (30)).
§ This polymorphism occurs with P467S (table 2), and the haplotype is designated *CYP3A4*19*. When this polymorphism occurs absent P457S, it is designated *CYP3A4*1G* (24).

organ donors or normal portions of surgical specimens (27).

Further confirmation of the *CYP3A4*1B* variant was made when sequence determination for the 5′ flanking region and the entire coding region was performed for 20 Caucasians, 20 African Americans, and 20 Chinese (28). This strategy ensures a greater than 98.5 percent probability of finding any polymorphism that has a frequency of at least 0.10, as determined by using binomial probability (29). Consequently, this approach revealed several additional *CYP3A4* polymorphisms. At codon 222, an amino acid substitution serine/proline was observed (*CYP3A4*2*). Another rare allelic variant in codon 455, designated *CYP3A4*3*, was found in a single Chinese subject. A silent polymorphism was observed in African Americans; this polymorphism was apparently confirmed by Lamba et al. (30) by using direct DNA sequencing. These authors designated this polymorphism $CYP3A4^{I1193I}$, denoting a silent change in an isoleucine codon (table 2).

When polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis was used, three more novel variants of *CYP3A4* were found in Chinese subjects

TABLE 4. Reported frequencies of *CYP3A4*1B*

Population (no.)	Genotype			F* (95% CI†)	Hardy-Weinberg equilibrium χ^2	p value	Study author(s) and year (reference no.)
	AA	AG	GG				
Caucasians (94)	79	12	3	0.096 (0.054, 0.138)	6.49	0.011	Rebbeck et al., 1998 (26)
Prostate cancer (230)	188	42‡					
African Americans (70)	15	36	9	0.530 (0.447, 0.613)	0.714	0.789	Walker et al., 1998 (36)
Caucasians (132)	114	13	5	0.087 (0.053, 0.121)	19.15	10^{-5}	
Chinese (130)	130	0	0	0.000			
Caucasians§ (39)	36	3	0	0.038 (0, 0.081)	0.062	0.803	Westlind et al., 1999 (27)
Japanese (128)	128	0	0	0.000			Ando et al., 1999 (37)
Caucasians (273)	526/20¶			0.036 (0.020, 0.052)			Ball et al., 1999 (38)
Hispanics (188)	341/35¶			0.093 (0.064, 0.122)			
African Americans (186)	169/203¶			0.546 (0.495, 0.597)			
African Americans (90)	59/121¶			0.672 (0.603, 0.741)			
Chinese (78)	78	0	0	0.000			
Japanese (77)	77	0	0	0.000			
Asians (80)	80	0	0	0.000			Paris et al., 1999 (39)
Caucasians (117)	109	7	1	0.038 (0.013, 0.063)	4.273	0.039	
Hispanics (121)	97	22	2	0.107 (0.068, 0.146)	0.327	0.567	
African Americans (116)	22	62	32	0.543 (0.479, 0.607)	0.687	0.407	
African Americans/prostate cancer (174)	30	64	80	0.644 (0.594, 0.694)	6.832	0.009	
Caucasians (101)	90	11	0	0.054 (0.023, 0.085)	0.335	0.563	Tayeb et al., 2000 (43)
Saudis (101)	84	16	1	0.089 (0.050, 0.128)	0.059	0.808	
Ghanians (100)	13	36	51	0.690 (0.626, 0.754)	2.512	0.113	
African Americans (75)	6	38	31	0.667 (0.592, 0.742)	1.470	0.225	Sata et al., 2000 (28)
Caucasians (59)	54	5	0	0.042 (0.006, 0.078)	0.120	0.734	
Chinese (59)	59	0	0	0.000			
African Americans (15)	1	4	10	0.800 (0.657, 0.943)	0.416	0.519	Wandel et al., 2000 (44)
Caucasians (56)	45	11	0	0.196 (0.141, 0.251)	0.664	0.451	Tayeb et al., 2002 (82)
Caucasians/prostate cancer (28)	11	17	0	0.304 (0.184, 0.424)	4.797	0.021	

Table continues

(n = 102) (31). These alleles were designated *CYP3A4*4*, *CYP3A4*5*, and *CYP3A4*6*. When PCR with standard fluorescence-based DNA sequencing and DNA from a mixed population originating in a variety of institutions in the United States and the United Kingdom (32) was used, two more allelic variants in the promoter region designated *CYP3A4*1C* and *CYP3A4*1D* were found. Similarly, but by using a single stranded conformation polymorphism in addition to DNA sequencing in a geographically diverse population of Caucasians, Hamzeiy et al. (33) reported two additional promoter-region single nucleotide polymorphisms, *CYP3A4*1E* and *CYP3A4*1F*. This group also reported a nine nucleotide insertion between positions –844 and –845 in the *CYP3A4*15A* allele, subsequently designated *CYP3A4*15B* (table 1).

Seventeen more genetic variants were identified by Eiselt et al. (34). Seven resulted in amino acid substitutions, seven were in introns, two were silent in exons, and one was in the 3′UTR. All variants were single nucleotide polymorphisms discovered by PCR and DNA sequencing in samples originating from European and Middle Eastern populations. The amino acid substitutions were designated *CYP3A4*7*, *CYP3A4*8*, *CYP3A4*9*, *CYP3A4*10*, *CYP3A4*11*, *CYP3A4*12*, and *CYP3A4*13* (table 2). Silent DNA-sequence variants, and those in introns and the 3′UTR, were designated M10–M19 (tables 1, 2, and 3).

Using direct sequencing of genomic DNA from multiple ethnic groups, Dai et al. (35) identified 28 polymorphisms including two new ones in the 3′UTR (table 1) and three new coding-region polymorphisms, *CYP3A4*17*, *CYP3A4*18*, and *CYP3A4*19* (table 2). They also described two silent coding-region polymorphisms, one in exon 10 and one in exon 11 (table 2), as well as seven new polymorphisms in introns 4, 5, 7, and 9–11 (table 3). Note that *CYP3A4*19* describes the haplotype of the single nucleotide polymorphisms at positions 85273 and 82266 (tables 2 and 3, respectively).

Lamba et al. (30) used small groups of ethnically diverse samples (numbering between five and 10), as well as 21 African-American and 53 Caucasian subjects, to investigate the entire gene by PCR-DNA sequencing. In addition to the

TABLE 4. Continued

Population (no.)	Genotype			*F* (95% CI)	Hardy-Weinberg equilibrium χ^2	*p* value	Study author(s) and year (reference no.)
	AA	AG	GG				
Caucasians (101)	90	9	2	0.064 (0.030, 0.098)	6.830	0.009	Garcia-Martin 2002 (40)
Caucasians (53)	99/7¶			0.065 (0.018, 0.112)			Lamba et al., 2002 (30)
African Americans (21)	22/20¶			0.480 (0.329, 0.631)			
Caucasians (340)	300	26	14	0.079 (0.061, 0.102)	77.355	$<10^{-6}$	Zeigler-Johnson et al., 2002 (45)
African Americans (130)	27	52	51	0.592 (0.532, 0.650)	3.836	0.050	
Ghanians# (118)	7	32	79	0.810 (0.750, 0.851)	2.180	0.140	
Senegalese# (173)	14	47	112	0.780 (0.737, 0.823)	6.913	0.009	
Caucasians (Australia)							Spurdle et al., 2002 (80)
Controls (breast cancer)	468	31	1	0.033 (0.022, 0.044)	0.408	0.523	
Breast cancer	899	52	0	0.027 (0.020, 0.035)	0.751	0.386	
Controls (ovarian cancer)	260	15	1	0.031 (0.016, 0.045)	2.217	0.136	
Ovarian cancer	449	38	1	0.041 (0.029, 0.053)	0.043	0.835	
Caucasians							Tayeb et al., 2003 (83)
Benign prostatic hyperplasia (379)	344	35	0	0.046 (0.031, 0.061)	0.888	0.346	
Benign prostatic hyperplasia/prostate cancer (21)	16	4	1	0.143 (0.034, 0.251)	1.037	0.309	
Caucasians**							Plummer et al., 2003 (25)
Prostate cancer (390)	362	24	4	0.041 (0.027, 0.055)	0.082	0.774	
Controls (426)	391	34	1	0.042 (0.028, 0.056)	18.519	$<10^{-4}$	
African Americans**							Plummer et al., 2003 (25)
Prostate cancer (38)	11	13	14	0.539 (0.427, 0.652)	0.707	0.401	
Controls (38)	8	16	14	0.579 (0.468, 0.690)	3.687	0.055	
Japanese							Fukushima et al., 2004 (24)
Hospital patients (416)	416	0	0	0.000			

* *F*, variant allele frequency.
† CI, confidence interval.
‡ Heterozygote and variant homozygotes were collapsed to compare prostate cancer cases with controls.
§ No specific details of race/ethnicity were given; samples were collected at surgery or autopsy at a hospital in Gothenburg, Sweden.
¶ Numbers in these lines are alleles (A/G); it was impossible to deduce full genotypes. Consequently, Hardy-Weinberg equilibrium was also unavailable.
# In the original publication (45), Africans from modern-day Africa were further divided into tribal groups.
** Sibling-pair case-control design (the Caucasian control group may contain related persons).

polymorphisms described above, they presented data for 20 additional polymorphisms or mutations: five were in the 5′UTR/promoter region, three were in the 3′UTR, and three were in coding regions (tables 1 and 2). The rest were found in introns 2–4, 7, and 10 (table 3). As of this writing (May 3, 2004), all of the known 78 individual, constitutive DNA sequence variants (inherited polymorphisms or mutations) of *CYP3A4* are listed in tables 1, 2, and 3.

Genotypes and allele frequencies. Genotypes and allelic frequencies for *CYP3A4*1B*, the minor variant of the original *CYP3A4* promoter-region polymorphism, are given in table 4. The frequency of this allele varies greatly between different ethnic/racial populations. It has been found to be the major variant among people of African origin, but six studies have failed to find this variant in Chinese, Taiwanese, or Japanese (24, 28, 36–39). The allelic frequency of *CYP3A4*1B* has been found to be 0.036–0.096 in Caucasians, 0.480–0.800 in African Americans, 0.093–0.107 in Hispanics, 0.089 in Saudis, and 0.690 in Ghanaians (26, 27, 40–43) (table 4). However, one small study of 15 European Americans did not find the minor variant (44).

The allelic frequency of *CYP3A4*1B* has been the most extensively studied (table 4). Fourteen studies examined *CYP3A4*1B* variants in nondiseased or healthy Caucasian populations; in five of these studies, the genotypic distribution did not conform to Hardy-Weinberg population laws (26, 36, 39, 40, 45). In each case, the reason for this finding was an excess number of *CYP3A4*1B* variant homozygotes. There could be several reasons, including faulty genotyping; however, the underlying basis is obscure and is not discussed in the original articles. In two of three prostate cancer populations for which the Hardy-Weinberg chi-square statistic could be calculated, highly significant deviation from Hardy-Weinberg equilibrium was observed (table 4). In the African-American population, a large excess of *CYP3A4*1B* variant homozygotes was observed. In the Caucasian population, although no homozygotes were observed, there was an excess of heterozygotes. When a specific allele is associated with disease, as may

TABLE 5. Reported frequencies of nonsynonymous polymorphisms of *CYP3A4*

Allele	Population (no.)	Genotype			F* (95% CI†)	Hardy-Weinberg equilibrium χ^2	p value	Study author(s) and year (reference no.)
		pp	pq	qq				
*CYP3A4*2*	Caucasians (55)	52	3	0	0.027 (0, 0.006)	0.040	0.835	Sata et al., 2000 (28)
*CYP3A4*3*	Caucasians (213)	211	2	0	0.006 (0, 0.010)	0.005	0.945	Eiselt et al., 2001 (34)
*CYP3A4*4*	Chinese (102)	99	3	0	0.017 (0, 0.031)	0.023	0.880	Hsieh et al., 2001 (31)
*CYP3A4*5*	Chinese (102)	100	2	0	0.014 (0, 0.023)	0.010	0.920	Hsieh et al., 2001 (31)
*CYP3A4*6*	Chinese (102)	101	1	0	0.005 (0, 0.014)	0.002	0.960	Hsieh et al., 2001 (31)
	Japanese (416)	415	1	0	0.001 (0, 0.004)	0.001	0.980	Fukushima et al., 2004 (24)
*CYP3A4*7*	Caucasians (213)	207	6	0	0.011 (0.003, 0.025)	0.040	0.835	Eiselt et al., 2001 (34)
*CYP3A4*8*	Caucasians (150)	149	1	0	0.007 (0, 0.010)	0.002	0.967	Eiselt et al., 2001 (34)
*CYP3A4*9*	Caucasians (213)	212	1	0	0.005 (0, 0.007)	0.001	0.973	Eiselt et al., 2001 (34)
*CYP3A4*10*	Caucasians (213)	212	1	0	0.005 (0, 0.007)	0.001	0.973	Eiselt et al., 2001 (34)
	Caucasians (53)	Unavailable			0.020			Lamba et al., 2002 (30)
*CYP3A4*11*	Caucasians (149)	148	1	0	0.007 (0, 0.010)	0.002	0.967	Eiselt et al., 2001 (34)
*CYP3A4*11b*	Japanese (416)	415	1	0	0.001 (0, 0.004)	0.001	0.980	Fukushima et al., 2004 (24)
*CYP3A4*11c*	Japanese (416)	415	1	0	0.001 (0, 0.004)	0.001	0.980	Fukushima et al., 2004 (24)
*CYP3A4*12*	Caucasians (149)	148	1	0	0.007 (0, 0.010)	0.002	0.967	Eiselt et al., 2001 (34)
*CYP3A4*13*	Caucasians (149)	148	1	0	0.007 (0, 0.010)	0.002	0.967	Eiselt et al., 2001 (34)
*CYP3A4*14*	Unknown‡ (8)	7	1	0				Lamba et al., 2002 (30)
*CYP3A4*15*	African Americans (21)	20	1	0	0.024 (0, 0.070)	0.012	0.911	Lamba et al., 2002 (30)
	African Americans (24)	22	2	0	0.042 (0, 0.982)	0.045	0.831	Dai et al., 2001 (35)
*CYP3A4*16*	Mexicans (10)	9	1	0	0.050 (0, 0.146)	0.028	0.868	Lamba et al., 2002 (30)
	Japanese (10)	9	1	0	0.050 (0, 0.146)	0.028	0.868	Lamba et al., 2002 (30)
*CYP3A4*16B*	Japanese (416)	405	11	0	0.013 (0.005, 0.021)	0.075	0.785	Fukushima et al., 2004 (24)
*CYP3A4*17*	Caucasians (24)	23	1	0	0.021 (0, 0.061)	0.011	0.917	Dai et al., 2001 (35)
*CYP3A4*18*	Asians (24)	23	1	0	0.021 (0, 0.061)	0.011	0.917	Dai et al., 2001 (35)
*CYP3A4*18B*	Japanese (416)	394	21	1	0.028 (0.017, 0.039)	1.548	0.213	Fukushima et al., 2004 (24)
*CYP3A4*19*	Asians (24)	23	1	0	0.021 (0, 0.061)	0.011	0.917	Dai et al., 2001 (35)

* *F*, variant allele frequency.
† CI, confidence interval.
‡ One chromosome of 16 of an eight-sample set procured from the Coriell Institute. A zero frequency was found for all other samples studied.

be the case with the *CYP3A4*1B* variant, deviation from Hardy-Weinberg equilibrium among cases is not unexpected.

Almost all of the other DNA-sequence variants described fall into the category of rare polymorphisms (frequency = 0.01–0.03) or mutations (frequency < 0.01). Many of the populations in which these variants were found were inadequate to properly determine genotypic and allelic frequencies (tables 5 and 6). Examples of inadequate populations for these purposes are those of mixed descent (33) or those with very small sample sizes (30). The frequencies of nonsynonymous coding region *CYP3A4* DNA-sequence variants are given in table 5. There are 22, of which nine are technically mutations. In addition, confirmation in a robust, independent population is needed for most of these alleles (24, 28, 30, 31, 34, 35).

Several non-coding-region polymorphisms were reported with their allelic frequencies, but genotyping data were generally not available. In two studies of African Americans (n = 24 and n = 21), nine polymorphisms of moderately high frequency were found in intron 2 (T/C, 5917, 0.146), exon 7 (*CYP3A4*^{I193I}, 0.05; also previously reported to be 0.046 by Sata et al. (28)), intron 7 (T/G, 15753, 0.48 and 0.50; T/C, 15783, 0.087 and 0.05; T/A, 15837, 0.065), intron 10 (G/A, 20230, 0.73 and 0.50; C/T, 20265, 0.083; G/C, 20309, 0.104 and 0.05), and intron 11 (C/T, 23081, 0.21 and 0.15) (table 6) (30, 35). Among 53 Caucasians, two polymorphisms of moderately high frequency were found in intron 7 (T/G, 15753, 0.065) and intron 10 (G/A, 20230, 0.11) (30). The frequency of the intron 10 polymorphism in Caucasians was also reported to be 0.095 and 0.146 by Eiselt et al. (34) and Dai et al. (35), respectively. The frequency of this polymorphism in Asians was reported to be 0.375 (35).

A recent report (24) described the frequency of 24 *CYP34A* DNA-sequence variants among 416 Japanese using PCR-DNA sequencing. Seven were already known (76236, 79698^9, 82266, *CYP3A4*6*, *11*, *16*, and *18*), and most were mutations (the frequency of 20 of the variants was <0.01, 95 percent confidence interval (CI): 0, 0.008). In addition, another recent report described a TGT insertion in CLEM 4, an enhancer of *CYP3A4* (46). This TGT insertion was described as being located approximately 11 kilobases to the 5′ of the *CYP3A4* ATG site.

TABLE 6. Allelic frequency* of CYP3A4 noncoding polymorphisms other than *CYP3A4*1B*

Allele	Population (no.)	Genotype			*F* (95% CI†)	Hardy-Weinberg equilibrium χ^2	*p* value	Study author(s) and year (reference no.)
		pp	pq	qq				
T5922C	African Americans (24)	Unavailable			0.146			Dai et al., 2001 (35)
*CYP3A4^I193I^*‡	African Americans (53)	49	5	0	0.046 (0.007, 0.086)	0.127	0.721	Sata et al., 2000 (28)
	African Americans (21)	Unavailable			0.05			Lamba et al., 2002 (30)
T15871G	African Americans (24)	Unavailable			0.50			Dai et al., 2001 (35)
	Caucasians (53)	Unavailable			0.065			Lamba et al., 2002 (30)
	African Americans (21)	Unavailable			0.48			Lamba et al., 2002 (30)
T15901C	African Americans (24)	Unavailable			0.087			Dai et al., 2001 (35)
	African Americans (21)	Unavailable			0.05			Lamba et al., 2002 (30)
T15955A	African Americans (24)	Unavailable			0.065			Dai et al., 2001 (35)
G20338A	Caucasians (148)	Unavailable			0.095			Eiselt et al., 2001 (34)
	African Americans (24)	Unavailable			0.73			Dai et al., 2001 (35)
	Caucasians (24)	Unavailable			0.146			Dai et al., 2001 (35)
	Asians (24)	Unavailable			0.375			Dai et al., 2001 (35)
	Caucasians (53)	Unavailable			0.110			Lamba et al., 2002 (30)
	African Americans (21)	Unavailable			0.50			Lamba et al., 2002 (30)
SNP16	African Americans (24)	Unavailable			0.083			Dai et al., 2001 (35)
G20417C	African Americans (24)	Unavailable			0.104			Dai et al., 2001 (35)
	African Americans (21)	Unavailable			0.05			Lamba et al., 2002 (30)
C23187T	African Americans (24)	Unavailable			0.210			Dai et al., 2001 (35)
	African Americans (21)	Unavailable			0.15			Lamba et al., 2002 (30)
*CYP3A4*1G*	Japanese (416)	281	114	21	0.188 (0.163, 0.212)			Fukushima et al., 2004 (24)
*CYP3A4*1H*	Japanese (416)	408	8	0	0.01 (0.003, 0.016)			Fukushima et al., 2004 (24)
*CYP3A4*1J*	Japanese (416)	413	3	0	0.004 (0, 0.008)			Fukushima et al., 2004 (24)
*CYP3A4*1K*§	Japanese (416)	415	1	0	0.001 (0, 0.004)			Fukushima et al., 2004 (24)

* Polymorphisms with a variant allele frequency (*F*) of <0.05 or a frequency determined in mixed populations were excluded.

† CI, confidence interval.

‡ Silent or synonymous single nucleotide polymorphism.

§ Sixteen additional DNA-sequence variants were discovered with a low frequency and were designated *CYP3A4*1K*, **1L*, **1M*, **1N*, **1P*, **1Q*,, **1R*, **1S*, **1T*, **1u*, **1v*, **1w*, **1x*, **1y*, **1z*, and **1aa*. Lowercase designations indicate potentially ambiguous haplotypes. The frequency of *CYP3A4*A* was found to be 0.734 (0.704, 0.764) in this Japanese population.

DISEASE BURDEN

Breast cancer is the second leading cause of death in women. There were 211,300 new cases of breast cancer in the United States in 2003 and 40,000 deaths from the disease (47). Several established risk factors for breast cancer include family history of breast cancer, early menarche, late age at first birth and nulliparity, and genetic factors (*BRCA1* and *BRCA2*). Other factors have been strongly implicated but are less well established, including oral contraceptive use, hormone replacement therapy, low physical exercise levels, and obesity (48). Most of these factors point toward increased exposure to estrogen. Several epidemiologic studies have shown that allelic variation in other genes such as *p53* may render a woman susceptible to breast cancer (49–53). Few studies have investigated a role for *CYP3A4* in breast cancer risk.

Prostate cancer is the most common non-skin-related cancer affecting men in the United States and the second leading cause of cancer-related deaths (54). An estimated 220,900 new cancer cases and an estimated 28,900 deaths were expected for 2003 (47). Major risk factors for prostate cancer are less well defined than those for breast cancer. There is compelling evidence of family history for prostate cancer (55); other major risk factors include race (the disease is common in Caucasians and African Americans but not Africans, rare in Asians) and age. Other possible risk factors include diet, multiple sexual partners, urban environment, body mass index, physical activity, vasectomy, and hormonal factors (56). These observations imply that different lifestyle factors are important, and, given that factors that increase estrogen levels and reduce testosterone levels are protective, it is probable that exposure to testosterone is important.

Several genes have been identified as accounting for some of the approximately 9 percent of prostate cancers that are familial. *RANSEL* is an interferon-inducible endoribonuclease linked to *HPC1*, which is also associated with familial prostate cancer. Whereas mutations have been identified for *RANSEL*, *HPC1* was found as the result of a genome-wide scan. Other genes with prostate-cancer-associated mutations

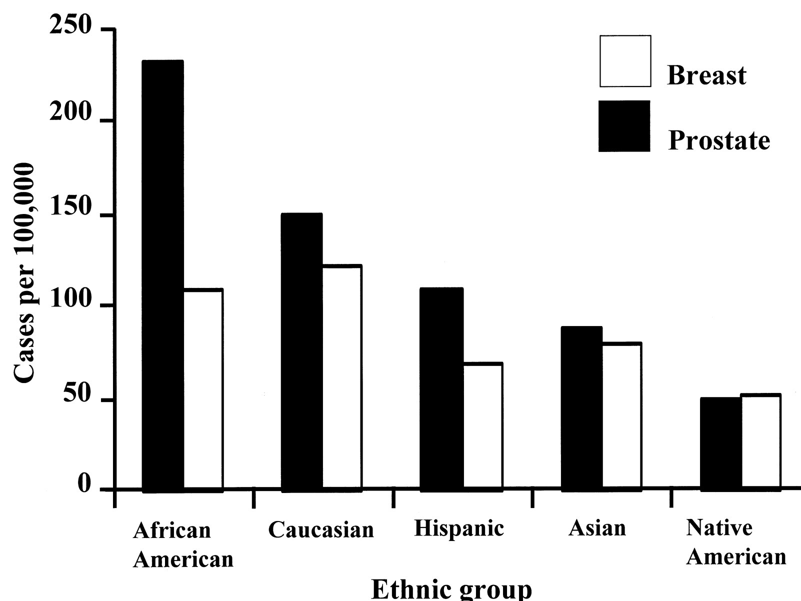

FIGURE 3. Incidence rates of breast and prostate cancer in five different ethnic groups, Surveillance, Epidemiology, and End Results Program, 1992–1998. Frequencies of the *CYP3A4*1B* variant: 0.480–0.817 among African Americans, 0.036–0.096 among Caucasians, 0.093–0.107 among Hispanics, 0.000 among Asians, and unknown among Native Americans. The population frequencies of *CYP3A4*1B* are roughly correlated with prostate cancer incidence ($r = 0.9$, $p < 0.05$, df = 2) but are poorly correlated with breast cancer incidence ($r = 0.6$, $p > 0.1$, df = 2).

include *MSR1* and *ELAC2* (56). Polymorphisms in canonical repeat regions of both *AR* and *SRD5A2* also are thought to increase prostate cancer susceptibility, and a *CYP17* (steroid 17-lyase) polymorphism may also be involved (figure 2) (57–64).

Interindividual and interethnic variability in the expression of *CYP3A4* accounts for large differences in disposition to xenobiotics, therapeutic drugs, and endobiotics (steroids) such as the oxidation of testosterone and the hydroxylation of estrogens. This difference in disposition to xenobiotics and drugs has been found to be at least 10–40-fold (9, 14, 27, 30, 65–69). Interestingly, population distributions of both rates of steroid hydroxylation (6β-hydroxylation of testosterone pmol/mg per minute) and metabolite:substrate ratio (e.g., 6β-hydroxycortisol/cortisol) appear to be unimodal (69). This study was performed before *CYP3A4* polymorphisms were discovered, but several studies have addressed the question of *CYP3A4* polymorphism-activity relations (27, 28, 31, 34, 35, 38, 40, 44). Two in vivo studies, using erythromycin and midazolam as probe drugs, failed to find any activity relations with *CYP3A4*1B*, **2*, **4*, **5*, **6*, **8*, **11*, **12*, or **13* (38, 40). A third study using midazolam noted a modest reduction in clearance (30 percent, $p = 0.02$) associated with *CYP3A4*1B* (44). Two in vitro studies of testosterone hydroxylase found no activity relations with *CYP3A4*1B*, **3*, **7*, **8*, **9*, **10*, **11*, **12*, or **13* (27, 31). A third study, which additionally used clorpyrifos, found decreased activity with *CYP3A4*17*, increased activity with *CYP3A4*18*, and no associations with *CYP3A4*3* or **19* (35). A fourth study, which also used nifedipine, found altered enzyme kinetics associated with *CYP3A4*2* (higher K_m and lower V_{max}) but no overall intrinsic difference in clearance characteristics from *CYP3A4*1A* (28). Taken together, these data suggest a lack of evidence to date that the major polymorphic variants in *CYP3A4* have any association with CYP3A4 activity.

With this information in mind, it does not logically follow that *CYP3A4* polymorphisms would be associated with steroid metabolism related to breast and prostate cancer. However, the original polymorphism association study of prostate cancer was published before the phenotyping studies (26). Moreover, the rate of prostate cancer in African Americans (230/105) is higher than that in Caucasians (150/105), which is higher than the rate in Asians (80/105) (figure 3). This trend roughly correlates with the frequency of the promoter-region *CYP3A4*1B* variant in these populations (table 4), potentially accounting for the fact that African-American men present with more severe forms of prostate cancer, possibly leading to a more aggressive course of the disease (70, 71).

Breast cancer

CYP3A4 plays a major role in the 4- and 16α-hydroxylation of estrogens, particularly estrone, the predominant form of estrogens in postmenopausal women (16, 72–76). This enzyme, in conjunction with the action of CYP1A2, which catalyzes the formation of 2-hydroxyestrone, may determine 2-hydroxyestrone:16α-hydroxyestrone ratios that in turn may be associated with breast carcinogenesis (77, 78).

Evidence suggests that overexpression of *CYP3A4* may be associated with breast cancer. To determine which members of the cytochrome P-450 superfamily are expressed in human breast tissue and tumors, Huang et al. (13) studied the

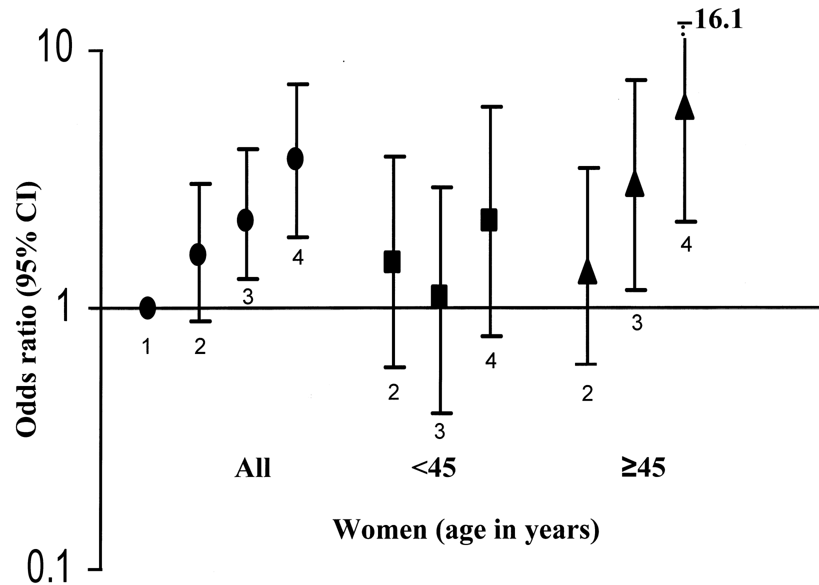

FIGURE 4. Possible involvement of CYP3A4 in estrogen metabolism and breast cancer risk in Chinese women. Data were plotted from those presented by Zheng et al. (79). Odds ratios and 95% confidence intervals (CIs) are given for quantities of urinary 6β-hydroxycortisol:cortisol ratios; 1 is the lowest quintile (referent), and 4 is the highest. Circles, data for all women; squares, data for younger women; triangles, data for older women.

mRNA expression levels. CYP3A4 mRNA was present in 70 percent of normal breast tissues and 18 percent of tumor tissues; however, the sample size was very small (13 for normal tissue, 11 for tumor tissue).

Zheng et al. (79) evaluated the association between urinary cortisol ratios and breast cancer risk in a subgroup of women who participated in a population-based case-control study in Shanghai, China. They found a strong association between the risk of breast cancer and urinary 6β-hydroxycortisol: cortisol ratios, where the metabolite:steroid ratio is a surrogate measure of CYP3A4 activity. They further showed that the risk increased in a dose-response manner. This association appeared to be driven by cortisol metabolism in older women (figure 4). Taken together, these investigations provided some impetus for recent genotyping studies of *CYP3A4* in relation to breast cancer risk.

There are two known genotyping studies of *CYP3A4* and breast cancer or breast carcinogenesis (80, 81): one is a molecular epidemiologic case-control study, but the other links genotype and breast cancer risk factors in a small group of healthy, young women. Details of these studies are given in tables 7 and 8. In the case-control study of Australian, Caucasian women, *CYP3A4*1B* was not associated with breast cancer (odds ratio (OR) = 0.86, 95 percent CI: 0.54, 1.33) or ovarian cancer (OR = 1.51, 95 percent CI: 0.80, 2.89) (80) (table 7). This conclusion did not change when the data were stratified by age (<40/≥40 years) or menopausal status. However, in a group of US girls (n = 137; 39 African American, 57 Hispanic, and 41 Caucasian) early-onset menarche, a breast cancer risk factor, was associated with inheritance of the *CYP3A4*1B* allele (odds trend = 3.21, 95 percent CI: 1.62, 6.89) (81).

There is evidence for a correlation between *CYP3A4*1B* and early life events associated with breast cancer risk, but one case-control study failed to find an association between breast cancer and *CYP3A4*1B* (80). These data suggest that, to address this question further, future case-control study designs should specifically and rigorously include issues of estrogen exposure to tease out what might be a small, but significant risk factor.

Prostate cancer

Differential steroid metabolite levels may result from *CYP3A4* polymorphisms, and CYP3A4 is involved in the oxidation of testosterone to 2β-, 6β-, or 15β-hydroxytestosterone. Potential interindividual variation in CYP3A4 function may play a role in androgen-mediated prostate carcinogenesis if the bioavailability of testosterone is affected, because steroid 5α-reductase converts testosterone to dihydrotestosterone, which mediates prostate cell growth (figure 2).

To our knowledge, there are five molecular epidemiologic studies of prostate cancer (25, 26, 39, 82, 83). Two are case-only studies linking clinical characteristics of disease with genotype, two are prospective studies of high-risk men (benign prostatic hyperplasia cases), and one is a case-sibling control study. Details of these studies, including genotyping methods used, are given in tables 7 and 8.

The first genotyping study known to approach this question included the discovery of the promoter-region variant *CYP3A4*1B* (26). In this case-only study, association between a variety of clinical characteristics of prostate cancer and inheritance of *CYP3A4*1B* was investigated. For

TABLE 7. Results of case-control and cohort studies of breast and prostate cancer

Population	Cases (no.)	Controls (no.)	OR*	95% CI*	Study author(s) and year (reference no.)
Breast cancer					
Caucasians (Australia)	951	500	0.86	0.54, 1.33	Spurdle et al., 2002 (80)
Prostate cancer					
Caucasians (United States)	230	94	1.17	0.62, 2.24†	Rebbeck et al.,1998 (26)
Case-only analysis	230				
Advanced stage			2.10	1.09, 4.05	
No family history of prostate cancer			2.72	1.24, 5.61	
>63 years of age			6.70	2.54, 35.17	
African Americans (United States)	174	116	1.12‡	0.61, 2.06	Paris et al., 1999 (39)
Case-only analysis	174				
Advanced stage			2.4	1.1, 5.4	
Caucasians (Scotland)§	28	56	6.32	2.31, 17.27	Tayeb et al., 2002 (82)
Caucasians (Scotland)§	21	344	2.7	0.77, 7.66	Tayeb et al., 2003 (83)
Caucasians (United States)¶	390	426	0.89	0.58, 1.44	Plummer et al., 2003 (25)
Advanced stage			1.91	1.02, 3.57	
African Americans (United States)¶	38	38	1.00	0.39, 2.54	
Advanced stage			0.49	0.17, 1.43	

* OR, odds ratio; CI, confidence interval.

† May not be a valid comparison because Hardy-Weinberg equilibrium $\chi^2 = 6.49$ and $p = 0.011$.

‡ For *CYP3A4*1B* homozygotes vs. the other genotypes, the odds ratio was significant (OR = 2.23, 95% CI: 1.35, 3.70).

§ Cohort studies of prostate cancer in men with benign prostate hypertrophy (OR = relative risk).

¶ Family case-control study (sibling controls).

230 incident, non-Hispanic, Caucasian prostate cancer cases recruited in the United States, the sequence context in the region of the nifedipine-specific responsive element was investigated by using a PCR-conformation sensitive gel electrophoresis method. Although the method was capable of distinguishing heterozygotes from homozygotes, all carriers of the *CYP3A4*1B* allele were collapsed into a single group, likely because homozygotes were somewhat infrequent. Here, we compared the *CYP3A4*1B* carrier frequencies between the 230 cases and the 94 healthy, unrelated Caucasians that Rebbeck et al. (26) referred to as a "reference panel" and found no risk of prostate cancer associated with carrier status (OR = 1.17, 95 percent CI: 0.62, 2.24) (table 7). Furthermore, the Hardy-Weinberg chi-square statistic for the putative control group was highly significant ($\chi^2 = 6.49$, $p = 0.011$). Therefore, it is possible that this comparison is invalid, although the mean age of each group was similar (63.3 vs. 63.4 years).

In the context of the case-only study, the focus was on comparison of *CYP3A4*1B* carrier status with the clinical attributes of prostate cancer, including age at diagnosis, prostate-specific antigen status, combined tumor grade (Gleason) and tumor-lymph node-metastasis, and family history (26). These analyses revealed that carriers of *CYP3A4*1B* were more likely to have tumors of a higher stage and grade. This effect was found to be greatest in older patients (diagnosed after 63 years of age) and in patients without a family history of the disease. The relative risk of advanced tumor stage associated with inheritance of *CYP3A4*1B*, when adjusting for detection method and age in a logistic regression model, was 2.1 (95 percent CI: 1.1, 4.1). The adjusted odds ratio for those patients with no family history of prostate cancer increased to 2.7 (95 percent CI: 1.2, 5.6). For those patients diagnosed at a later age (>63 years), the odds ratio increased to 6.7 (95 percent CI: 2.5, 17.7); for the older patients (>63 years of age) with no such family history, it was 9.5 (95 percent CI: 2.5, 35.2). No association was found between inheritance of *CYP3A4*1B* and prostate-specific antigen at the time of diagnosis, and there was no association with family history of cancer.

Subsequently, a functional role for *CYP3A4*1B* was investigated, and initial data suggested that higher levels of CYP3A4 expression are associated with it versus the *CYP3A4*1A* allele (37, 42). However, later mechanistic studies have not supported this link (83). In other studies, although amounts of CYP3A4 protein in liver microsomes have been observed to vary by race, no single *CYP3A4* allele has been significantly linked with the CYP3A4 phenotype (9, 27, 28, 31, 34, 35, 38, 40, 83).

Paris et al. (39) evaluated the *CYP3A4*1B* genotype frequencies in 174 African-American prostate cancer cases and 116 healthy volunteers (table 7). When these groups were compared, the crude odds ratio for *CYP3A4*1B*

TABLE 8. Details concerning populations of studies cited and laboratory methods

Study author(s) and year (reference no.)	Geographic location*	Period of study	Selection criteria	Genotyping method
Rebbeck et al., 1998 (26)	Philadelphia, Pennsylvania	1994–1997	Caucasian men (23–89 years of age), prostate cancer cases (45–90 years of age)	PCR†-CSGE†
Walker et al., 1998 (36)	Philadelphia, Pennsylvania; Taiwan	N/A†	Healthy, unrelated persons	PCR-CSGE
Westlind et al., 1999 (27)	Gothenberg, Sweden	N/A	Liver resection (in cancer cases)	PCR-DNA sequence
Ando et al., 1999 (37)	Japan	N/A	Healthy, unrelated persons	PCR-RFLP† (MboII)
Ball et al., 1999 (38)	United States	N/A	Healthy, unrelated persons	PCR-DNA sequence
Paris et al., 1999 (39)	Southern California	N/A	Healthy persons	TaqMan‡
	Cleveland Clinic, Cleveland, Ohio	1993–1998	Tissue blocks (with PCRable DNA)	
Tayeb et al., 2000 (43)	Scotland	N/A	Healthy, unrelated persons	PCR-SSCP†
Sata et al., 2000 (28)	Finland, Taiwan, United States	N/A	N/A	PCR-DNA sequence
Wandel et al., 2000 (44)	United States	N/A	Healthy, unrelated men (26–49 years of age)	TaqMan
Tayeb et al., 2002 (82)	Grampian region, Scotland	1974–1990	Men with benign prostatic hyperplasia whose initial biopsy was negative for prostate cancer and whose second biopsy, after 6 years, was positive for prostate cancer	PCR-SSCP
Garcia-Martin et al., 2002 (40)	Spain	N/A	Healthy, unrelated persons	PCR-DNA sequence
Lamba et al., 2002 (30)	Coriel Institute, Camden, New Jersey (DPDR†), large number of countries listed	N/A	Unrelated persons	PCR-DNA sequence
Zeigler-Johnson et al., 2002 (45)	Philadelphia, Pennsylvania (HUP†); Senegal; Ghana	1994–2001	No prior cancer diagnosis and prostate cancer cases and controls recruited at HUP	PCR-RFLP (ScrFI)
Spurdle et al., 2002 (80)	Melbourne and Sydney, Australia	1992–1995 and 1996–2000	Women with a first primary breast cancer diagnosis at <40 years of age (1992–1995), extended to 59 years of age (1996–2000)	TaqMan
Tayeb et al., 2003 (83)	Grampian region, Scotland	1989–2000	Diagnosis of benign prostatic hyperplasia in 1989 and diagnosis of prostate cancer by 2000	PCR-SSCP
Plummer et al., 2003 (25)	Cleveland, Ohio	N/A	Prostate cancer with sibling controls	SNuPe§
Eiselt et al., 2001 (34)	Basel, Switzerland	N/A	Healthy volunteers and patients with a variety of diagnoses (including cancer, stroke, pancreatitis, ulcers)	PCR-DNA sequence
Hsieh et al., 2001 (31)	Han, Taiwan	N/A	Healthy volunteers and stroke patients	PCR-SSCP and PCR-RFLP (BsmI, HinfI, ClaI)
Dai et al., 2001 (35)	Coriel Institute, Camden, New Jersey (DPDR); large number of countries listed	N/A	N/A	Direct DNA sequence
Fukushima et al., 2004 (24)	Tokyo, Osaka, and Chiba, Japan	N/A	Hospital patients: epileptic, cardiac, and cancer	PCR-DNA sequence

* As closely as could be defined from the original reference material.

† PCR, polymerase chain reaction; CSGE, confirmation sensitive gel electrophoresis; N/A, not addressed; RFLP, restriction fragment length polymorphism; SSCP, single stranded conformation polymorphism; DPDR, DNA polymorphism discovery resource; HUP, Hospital of the University of Pennsylvania.

‡ Manufactured by Applied Biosystems, Inc., Foster City, California.

§ SNuPe (single nucleotide primer extension) is a primer-specific, PCR-based assay for single nucleotide polymorphism determination (Amersham Biosciences, Piscataway, New Jersey).

carriers was not significant (OR = 1.1, 95 percent CI: 0.6, 2.1). However, carriers of *CYP3A4*1B* homozygotes were at a significantly increased risk (OR = 2.2, 95 percent CI: 1.4, 3.7; tables 4 and 7). This result was also reflected by the fact that an excess of homozygotes was detected in the Hardy-Weinberg statistic ($\chi^2 = 6.8$, $p < 0.01$), and it was consistent with the initial study of US Caucasians (26). When a case-only design was used to examine the impact of inheriting *CYP3A4*1B*, it was found to be associated with advanced clinical characteristics. Men with *CYP3A4*1B* variant homozygotes were also more likely to present with a higher grade and stage of prostate cancer (OR = 1.7, 95 percent CI: 0.9, 3.4), and the association was even stronger for older men (>65 years of age) (OR = 2.4, 95 percent CI: 1.1, 5.4). These results are broadly consistent with those of Rebbeck et al. (26).

A *CYP3A4*1B* genotyping study was conducted in a group of 84 Scottish, Caucasian men; all had a diagnosis of benign prostatic hyperplasia and had been recruited prospectively with respect to a prostate cancer diagnosis (82). In this group, inheritance of *CYP3A4*1B* was associated with a greater than sixfold increase in risk (relative risk = 6.3, 95 percent CI: 2.3,

17.3; table 7) of developing prostate cancer over a period of 6–15 years. No *CYP3A4*1B* homozygotes were observed in this study group; however, heterozygotes occurred in excess in the case group (Hardy-Weinberg $\chi^2 = 4.8$, $p = 0.02$). In a second cohort study of 400 men with benign prostatic hyperplasia, for 21 men who developed prostate cancer, the relative risk associated with inheritance of *CYP3A4*1B* was 2.7 (95 percent CI: 0.77, 7.66) (table 7) (83).

A study of Caucasians ($n = 816$) and African Americans ($n = 76$), in which a family-based case-control design was used (sibling pairs: 440 cases and 480 controls), investigated the *CYP3A4*1B* and *CYP3A5*1* genotypes and the *CYP3A4*1B*/*CYP3A5*3* haplotype for associations with prostate cancer risk and tumor aggressivity (23). Consistent with findings from previous studies, those for comparison of *CYP3A4*1B* frequencies between prostate cancer cases and controls showed no simple associations (OR = 0.9, 95 percent CI: 0.6, 1.4 for Caucasians and OR = 1.0, 95 percent CI: 0.4, 2.5 for African Americans). However, when stratified by aggressivity, results indicated that *CYP3A4*1B* was a risk factor for more aggressive prostate cancer in Caucasians (OR = 1.9, 95 percent CI: 1.0, 3.6) but not African Americans (OR = 0.5, 95 percent CI: 0.2, 1.4). The *CYP3A5*1* variant was inversely associated with prostate cancer in Caucasians with less aggressive disease (OR = 0.4, 95 percent CI: 0.2, 0.8) but not in African Americans (OR = 0.9, 95 percent CI: 0.1, 8.7). The *CYP3A4*1B*/*CYP3A5*3* haplotype was positively associated with prostate cancer when data from African Americans and Caucasians were combined (OR = 2.9, 95 percent CI: 1.4, 6.2).

Overall, the data from the five prostate cancer studies described above do not provide convincing support for a direct role of *CYP3A4*1B* in prostate carcinogenesis (25, 26, 38, 60, 61). However, the apparent association of *CYP3A4*1B* with other factors such as age and clinical stages of disease will likely make this role difficult to define. In the studies from Scotland, *CYP3A4*1B* appears to play a role in high-risk (benign prostatic hyperplasia) patients. Two studies of Caucasians suggest an association with advanced disease only, whereas findings from studies of African Americans are inconsistent. A major gap is the dearth of robust, but basic case-control studies; future studies might consider gene-environment and gene-gene interactions in seeking an association between prostate cancer and *CYP3A4* polymorphisms.

INTERACTIONS

Although we know of no research that has been conducted to specifically evaluate interactions among *CYP3A4* variants, breast cancer, and other external factors, CYP3A4's role in the metabolism of exogenous chemicals makes it a likely candidate for gene-environment interactions. *CYP3A4* is present in mammary epithelial cells (9, 10, 12, 13) and is involved in activation of many environmental carcinogens, such as the polycyclic aromatic hydrocarbons, heterocyclic amines, aflatoxin, and nitrosamines (14, 85–88). Some have been shown to be mammary carcinogens in laboratory animals (89–93). Furthermore, ingestion of polycyclic aromatic hydrocarbons and heterocyclic amines and metabolism via CYP3A4 has also been shown to result in formation of carcinogen-DNA adducts in mammary tissue (94). On the basis of this type of inferential evidence, the potential for a gene-environment interaction in the mechanism of breast cancer does seem biologically plausible.

The apparent complexity of the associations of *CYP3A4*1B* with prostate cancer risk in relation to age, clinical factors, and family history suggests that interactions with other probable endogenous factors are required. Thus, *CYP3A4*1B* is probably not an independent risk factor.

LABORATORY TESTS

No standardized laboratory tests exist for *CYP3A4* genotyping. A variety of methods for determining *CYP3A4* genotype have been used and were referred to above, and they are listed in table 8. These methods include dideoxy-chain termination DNA sequencing, real time-PCR, PCR-restriction fragment length polymorphism, single stranded conformation polymorphism, single nucleotide primer extension (SNuPe; Amersham Biosciences, Piscataway, New Jersey), and conformation sensitive gel electrophoresis.

POPULATION TESTING

Currently, there is insufficient evidence implicating DNA-sequence variation in *CYP3A4* in the etiology of either prostate or breast cancer for population testing. Development of haplotyping methods that identify specific groups of these polymorphisms may help to resolve this question (95). However, considering the complexity of sex hormone metabolism, it is unlikely that a single nucleotide polymorphism in a single steroid metabolism gene will be sufficiently implicated to warrant genetic testing.

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APPENDIX

Internet sites

1. Human cytochrome P-450 allele nomenclature: http://www.imm.ki.se/CYPalleles/CYP3A4.html
2. GeneCards: http://bioinfo.weizmann.ac.il/cards-bin/carddisp?CYP3A4
3. Cytochrome P-450 home page: http://drnelson.utmem.edu/CytochromeP450.html)
4. GenAtlas: http://www.dsi.univ-paris5.fr/genatlas/
5. Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/htbin-post/Omim/getmim?search=CYP3A4
6. Genome Database (GDB): http://www.gdb.org/gdb-bin/genera/genera/hgd/Gene?!action=query&displayName=CYP3A4